IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of
Tsuneo YASUMA et al. : Docket No.: 2006_1537A
Serial No. 10/594,996 : Group Art Unit: 1625
Filed September 29, 2006 : Examiner: SOLOLA, TAOFIQ A
For: ALKOXYPHENYLPROPANOIC ACID DERIVATIVES

DECLARATION UNDER 37 CFR §1.132

Honorable Commissioner for Patents,
P.O. Box 1450
Alexandria, Virginia 22313-1450

Sir:

I, Naoyuki Kanzaki, declare:

That I am a citizen of Japan, whose full post office address is c/o Takeda Pharmaceutical Company Limited, Discovery Research Center, 17-85, Jusohonmachi 2-chome, Yodogawa-ku, Osaka-shi, Osaka 532-8686 Japan;

That my education and employment history is as follows:

Responsible Person of the Experiment: Naoyuki Kanzaki

Title: Research Head, Discovery Research Center, Takeda Pharmaceutical Company, Ltd.

year	University / Company	Department	job title etc.	research outline
1985	Osaka University, Graduate School of Engineering, Division of Fermentation Technology		graduation (master's degree)	
1985	Takeda Chemical Industry Ltd.	Central Laboratories, Fermentation Product Laboratory	entrance	engaged in research on culturing of inosine/guanosine-producing microorganism
1987	Takeda Chemical Industry Ltd.	Food Laboratory		engaged in research on culturing of inosine/guanosine-producing microorganism
1988	Takeda Chemical Industry Ltd.	Applied Technology Laboratory		engaged in research on culturing of inosine/guanosine-producing microorganism
1990	Takeda Chemical Industry Ltd.	Food Vitamin Laboratory		engaged in research on inosine/guanosine fermentation
1993	Takeda Chemical Industry Ltd.	Food Vitamin Laboratory	Chief	engaged in research on inosine/guanosine/beta-carotene fermentation
1994	Takeda Chemical Industry Ltd.	Production Technology Laboratory, Center of Biotechnology	Scientist	engaged in research on biotin fermentation
1995	Takeda Chemical Industry Ltd.	Biotechnology Research Laboratory	Scientist	engaged in research on compound screening
1997	Takeda Chemical Industry Ltd.	Biotechnology Research Laboratory	Assistant Research Head	engaged in research on compound screening
1998	Takeda Chemical Industry Ltd.	Biotechnology Research Laboratory	Research Head	engaged in research on compound screening
1999	Takeda Chemical Industry Ltd.	Pharmaceutical Developmental Research Division, Developmental 4th laboratory	Research Head	engaged in research on compound screening
2001	Takeda Chemical Industry Ltd.	Pharmaceutical Research Division, Discovery Research Center	Research Head	engaged in research on compound screening
2004	Takeda Pharmaceutical Company, Ltd. (Change of Company's English Title)	Pharmaceutical Research Division, Discovery Research Center	Research Head	engaged in research on compound screening

I now work in the Pharmaceutical Research Division, Discovery Research Center, and am engaged as research head in research on compound screening.

That I conducted the following experiments to verify the unexpected effect of the present invention;

That the following demonstrates such fact:

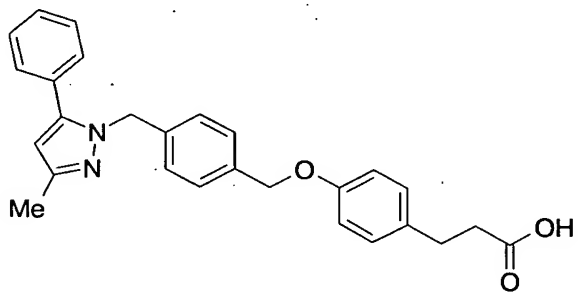
EXPERIMENTS

1. Object

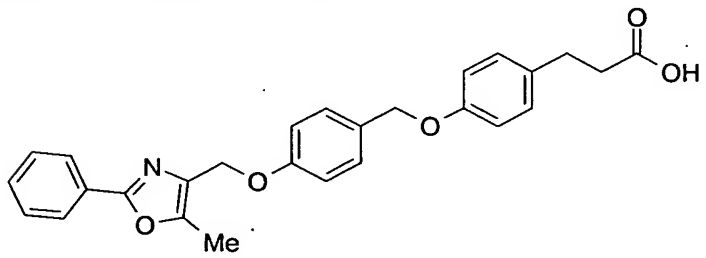
Evidence of the compound of the present invention having a superior GPR40 agonist activity than the compound of WO02/053547 is indicated in the experiments.

2. Test Compound

The present invention: Compound A



WO02/053547: Compound B



3. Test Method

Using AlphaScreen cAMP assay kit (PerkinElmer), the amount of intracellular cAMP was quantitated.

(1) A CHO (dhfr-) cell expressing human GPR40 was cultivated with an MEM-a culture medium comprising 10%

dialyzed serum (without nucleotide, Wako Pure Chemical Industries, Ltd. : 135-15175) at 37°C until it became nearly confluent.

(2) The above-mentioned cells were washed with PBS (Invitrogen), and the cells were detached with 0.5 mM EDTA. HBSS (Invitrogen: 4065056) comprising 5 mM HEPES (pH 7.4) was added thereto to count the number of cells. After centrifugation, the cells suspension were diluted with HBSS comprising 5 mM HEPES (pH 7.4) to make 2×10^6 cells/mL.

(3) The above-mentioned suspension (10 μL) was added to OptiPlate-384 (PerkinElmer) containing the diluted solution of the compound.

(4) The suspension was reacted at 37°C for 30 min.

(5) After the reaction, the amount of intracellular cAMP was quantitated using AlphaScreen cAMP assay kit (PerkinElmer, 60625). More specifically, a solution of dispersed Anti-cAMP Acceptor beads was added to each well, and then a mixed solution of Biotinylated-cAMP, reptavidine beads Donor beads and Tween-20 was added to dissolve the cells.

(6) After 5 hours in room temperature, Envision (PerkinElmer) was used for fluorescent measurement (Ex: 680 nm, Em: 520-620) to obtain the generated cAMP concentration from the standard curve which was prepared separately.

4. Results

The results are shown in the following Table 1.

Table 1

Compound	EC_{50}
compound A	57nM
compound B	250nM

5. Conclusion

It has been evidenced that the compound of the present invention has a superior GPR40 agonist activity than the compound of WO02/053547.

I further declare that all statements made herein are true to the best of my knowledge and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

Signed at Osaka, Japan, on this 13th day of May, 2008

Naoyuki Kanzaki

Naoyuki Kanzaki